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**Gut and liver T cells of common clonal origin are detected in primary  
sclerosing cholangitis-inflammatory bowel disease**

Eva Kristine Klemsdal Henriksen<sup>1,2,3,4</sup>, Kristin Kaasen Jørgensen<sup>1,5</sup>, Fatemeh Kaveh<sup>3,4,6</sup>, Kristian Holm<sup>1,2,3,4</sup>, David Hamm<sup>7</sup>, Johanna Olweus<sup>3,8,9</sup>, Espen Melum<sup>1,2,3,10</sup>, Brian K Chung<sup>1,4,13</sup>, Tor J Eide<sup>4,11</sup>, Knut EA Lundin<sup>10,12</sup>, Kirsten Muri Boberg<sup>1,3,4,10</sup>, Tom H Karlsen<sup>1,2,3,4,10</sup>, Gideon M Hirschfield<sup>13</sup>, Evaggelia Liaskou<sup>13</sup>

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## Abbreviations

PSC: primary sclerosing cholangitis; IBD: inflammatory bowel disease; FFPE: formalin-fixed, paraffin-embedded; TCR: T-cell receptor; gDNA: genomic DNA; CDR3: complementarity-determining region 3; V: variable, D: diversity; J: joining; HLA: human leukocyte antigen

## Keywords

high-throughput sequencing; human gut; human liver; IBD; PSC; T-cell receptor; ulcerative colitis

## Conflict of interest

Dr. Jørgensen has consulted and advised for Intercept Pharmaceuticals, Celltrion and Tillotts Pharma. Dr. Hamm owns stock and is employed by Adaptive Biotechnologies Corp. Dr. Hirschfield has consulted and advised for Intercept Pharmaceuticals, Lumena Pharmaceuticals, Janssen, GSK, NGM Bio, Dignity Sciences, and is on the speakers bureau for Falk Pharma. Dr. Hirschfield and Dr. Liaskou are supported by the NIHR Liver Biomedical Research Unit: the views expressed are those of the authors(s) and not necessarily those of the National Health System, the National Institute for Health Research, or the Department of Health. Remaining authors declare no conflicts of interest.

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### **Author contributions**

EKKH, KKJ, EM, THK, EL contributed to study design. KKJ, TJE, KL, KMB  
provided material support. EKKH performed HLA typing. EKKH and EL  
performed DNA extractions. FK prepared data for analysis. EKKH and KH  
performed data analysis. EKKH, KH, DH, JO, BKC, GH, TKH and EL  
interpreted the data and drafted the manuscript. All authors critically revised  
the manuscript and approved the final version.

## ABSTRACT

**Background & Aims:** Recruitment of gut-derived memory T cells to the liver is believed to drive hepatic inflammation in primary sclerosing cholangitis (PSC). However, whether gut-infiltrating and liver-infiltrating T cells share T-cell receptors (TCRs) and antigenic specificities is unknown. We used paired gut and liver samples from PSC patients with concurrent inflammatory bowel disease (PSC-IBD), and normal tissue samples from colon cancer controls, to assess potential T-cell clonotype overlap between the two compartments.

**Methods:** High-throughput sequencing of TCR $\beta$  repertoires was applied on matched colon, liver and blood samples from patients with PSC-IBD (n=10), and on paired tumor-adjacent normal gut and liver tissue samples from colon cancer patients (n=10).

**Results:** An average of 9.7% (range: 4.7–19.9%) memory T-cell clonotypes overlapped in paired PSC-IBD affected gut and liver samples, after excluding clonotypes present at similar frequencies in blood. Shared clonotypes constituted on average 16.0% (range: 8.7–32.6%) and 15.0% (range: 5.9–26.3%) of the liver and gut memory T cells, respectively. A significantly higher overlap was observed between paired PSC-IBD affected samples (8.7%,  $p=0.0007$ ) compared to paired normal gut and liver samples (3.6%), after downsampling to equal number of reads.

**Conclusion:** Memory T cells of common clonal origin were detected in paired gut and liver samples of patients with PSC-IBD. Our data indicate that this is related to PSC-IBD pathogenesis, suggesting that memory T cells driven by shared antigens are present in the gut and liver of PSC-IBD patients. Our findings support efforts to therapeutically target memory T-cell recruitment in PSC-IBD.

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Keywords: high-throughput sequencing; human gut; human liver; IBD; PSC;

T-cell receptor; ulcerative colitis



## INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic inflammatory liver disease of unknown etiology. Effective disease-modifying medical treatment remains elusive. PSC is strongly associated with inflammatory bowel disease (IBD), with 60–80% of PSC patients having coexistent IBD [1]. The mechanism responsible for this association is unclear, and patients may develop PSC after total colectomy, or IBD may appear after liver transplantation [2, 3]. A number of hypothesized pathways to liver injury have been suggested for PSC [1], and a variety of proposed phase II clinical trials target individual biological pathways [4].

Effector T cells homing to the intestine express high levels of  $\alpha 4\beta 7$  integrin and CCR9 chemokine receptor, which bind to the endothelial adhesion molecule MAdCAM-1 and CCL25 chemokine, respectively [5, 6]. It has been reported that there is an aberrant expression of the gut-specific molecules MAdCAM-1 and CCL25 in human PSC livers [7-9] and that  $\alpha 4\beta 7^+ \text{CCR9}^+$  effector memory T cells constitute approximately 20% of the liver-infiltrating lymphocytes in PSC patients [7]. These findings have led to the hypothesis that gut-derived memory T cells migrate to the liver and, upon recognition of an antigen, expand and promote inflammation [10]. In agreement, studies in mice show that T cells primed in the gut-associated lymphoid tissue by ovalbumin migrate to the liver and cause cholangitis when recognizing the same antigen on bile ducts [11]. In human PSC there has so far been no evidence for gut-infiltrating and liver-infiltrating T cells being clonally related. Using paired gut and liver tissue from PSC patients with concurrent IBD (PSC-IBD), we assessed whether gut-infiltrating and liver-infiltrating T cells

carry the same T-cell receptors (TCRs), *i.e.* are clonally related and able to recognize the same antigen(s) in the PSC-IBD affected gut and liver. We further investigated whether clonally related T cells are present in paired tumor-adjacent normal gut and liver tissue sampled from colon cancer patients.

## MATERIALS AND METHODS

### *Study populations*

Matched fresh-frozen explanted whole liver tissue (50–60mg), colonic biopsies from the ascending colon (1–5mg) and EDTA anti-coagulated peripheral blood (6ml) from Norwegian patients with PCS-IBD (n=10) were used in this study (see **Table 1** and **Supplementary Table 1** for clinical patient characteristics at time of each sample collection). PSC was diagnosed based on accepted criteria with typical findings of bile duct irregularities on cholangiography [12]. The diagnosis and classification of IBD was based on commonly accepted clinical, endoscopic and histopathologic criteria [13]. One patient was diagnosed with Crohn's disease, while the remaining nine patients had ulcerative colitis. Colonic biopsies had been collected during routine colonoscopy, and active colonic inflammation had been graded according to the Mayo UC endoscopic subscore [14, 15]. Blood samples had been collected at the same date as the colonic biopsies. The median time from the colonoscopy and blood sampling to liver transplantation was 3.5 years (range 0.6–7.3 years) (**Supplementary Table 1**). The study size was determined based on the number of available matched colonic biopsies, blood and liver samples from patients with PSC-IBD.

Paired tumor-adjacent normal gut and liver tissue were sampled in Birmingham, UK from patients with colon cancer that developed colorectal metastasis in their liver (n=10; see **Table 1** and **Supplementary Table 2** for clinical patient characteristics at time of each sample collection). The normal tissue was available as formalin-fixed, paraffin-embedded (FFPE) material.

## *Ethics*

The study was performed in accordance with the Declaration of Helsinki. The study was approved by local research ethics committees (2012-286 and 15/NW/0079), and written informed consent was obtained from all patients.

## *High-throughput sequencing and analysis of the TCR $\beta$ chain*

Genomic DNA (gDNA) was extracted from fresh-frozen PSC-IBD affected colonic biopsies and explanted whole liver tissue using the AllPrep DNA RNA micro and mini kit following the manufacturer's instruction (Qiagen, Valencia, CA, USA), respectively. Peripheral blood gDNA was extracted using a modified salting-out technique [16]. gDNA from FFPE normal gut and liver tissue was extracted using the QIAamp DNA FFPE tissue kit following the manufacturer's instruction (Qiagen). The rearranged TCR $\beta$  complementarity-determining region 3 (CDR3) was amplified from gDNA using a multiplex PCR and sequenced by Adaptive Biotechnologies (Seattle, WA, USA) using their ImmunoSEQ assay [17, 18]. Gut and liver TCR $\beta$  repertoires were sequenced at survey resolution, while peripheral blood TCR $\beta$  repertoires were sequenced at deep level resolution, according to company's guidelines for samples derived from non-lymphoid tissues and whole blood, respectively. PCR and sequencing errors were removed, and for each unique sequence, the nucleotide and predicted amino acid sequence, V (variable), D (diversity) and J (joining) genes and the number of sequencing reads were determined. The data was further sorted to exclude any sequence with an out-of-frame rearrangement or a stop codon in the CDR3, and the frequency was determined for each of the remaining productive unique sequences (nucleotide clonotypes).

Data were analysed using the ImmunoSEQ Analyzer (<http://www.adaptivebiotech.com/immunoseq/analyzer>), R statistical software environment (version 3.2.3, <https://www.R-project.org/>) and general bioinformatics strategies. T-cell clonality was calculated as  $1 - (\text{Shannon's Entropy}) / \log_2(\text{number of nucleotide clonotypes})$ . A clonality score of 1 indicates a monoclonal sample, whereas a score of 0 indicates a maximally diverse sample. The number of productive gene rearrangements was calculated as  $(\text{the number of sequencing reads}) / (\text{amplification factor})$ . The amplification factor of each sample was determined using spiked-in synthetic molecules. Richness was calculated as  $(\text{number of nucleotide clonotypes}) / (\text{number of productive gene rearrangements})$  and percent T cells was calculated as  $(\text{number of productive gene rearrangements}) / (\text{number of diploid genomes})$ . Considering the mass of a diploid genome is approximately 6.5pg, the number of diploid genomes was calculated using information on the mass of reaction template (the amount of gDNA used per sample). Nucleotide clonotype overlap was calculated as  $[(\text{number of nucleotide clonotypes found in both liver and gut} \times 2) / (\text{total number of nucleotide clonotypes in the liver and gut sample})] \times 100$ . Jaccard index was calculated using the 'sets' package [19] (version 1.0.16) in R as  $(\text{number of nucleotide clonotypes found in both liver and gut}) / ((\text{total number of nucleotide clonotypes in the liver and gut sample}) - (\text{the number of nucleotide clonotypes found in both liver and gut}))$ . The overlap coefficient was calculated as  $(\text{number of nucleotide clonotypes found in both liver and gut}) / (\text{total number of nucleotide clonotypes for the smallest patient sample})$ . The proportion of the liver and gut memory T cells carrying overlapping gut-liver memory clonotypes was calculated as

(number of sequencing reads for overlapping gut-liver memory clonotypes)/(total number of sequencing reads for memory TCR $\beta$  repertoire) X 100. 'Memory TCR $\beta$  repertoire' denotes here the gut or liver nucleotide clonotypes found at a frequency of >0.01%, and which were not detected in the matched blood sample at a frequency of >0.01%. For the comparison of clonotype overlap in paired PSC-IBD affected tissue samples and paired normal gut and liver tissue, each sample was downsampled to 20,264 reads.

### *HLA typing*

From peripheral blood gDNA of patients with PSC-IBD, *HLA-B* and *HLA-DRB1* were amplified and prepared for sequencing using the NGSgo workflow (GenDX, Utrecht, Netherlands). High-throughput sequencing of the samples was carried out on a MiSEQ instrument (Illumina, San Diego, CA, USA) by the Norwegian Sequencing Centre (Oslo, Norway), and the alleles were assigned using the NGSengine 1.7.0 software (GenDX).

### *Statistical analyses*

All values are presented as mean  $\pm$  SEM unless stated otherwise. Wilcoxon Signed Rank Test was used when comparing V and J gene usage of overlapping gut-liver memory clonotypes and the non-overlapping gut or liver memory clonotypes, while Mann-Whitney U test was used when comparing V and J gene usage of total gut and liver memory TCR $\beta$  repertoires, when comparing clonotype overlap in paired normal gut and liver tissue and paired PSC-IBD affected gut and liver tissue, when comparing V and J gene usage of nucleotide clonotypes in normal and PSC-IBD affected gut and liver tissues and to compare clonality scores. When comparing V and J gene usage, we

adjusted the p-values for the number of V and J genes tested using Benjamini-Hochberg procedure. A p-value less than 0.05 was regarded as statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22 software (IBM, Armonk, NY, USA) and R statistical software environment (version 3.2.3, <https://www.R-project.org/>), and graphs were made using Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

### *Gut and liver memory T cells of common clonal origin are detected in patients with PSC-IBD*

We sequenced the TCR $\beta$  repertoires of liver, gut and blood-derived T cells from 10 patients with PSC-IBD (**Table 1** and **Supplementary Table 1**). A detailed description of the TCR $\beta$  sequence data is given in **Supplementary Table 3**. The HLA type of each patient with PSC-IBD was also determined; all patients had at least one *HLA-DRB1* PSC risk allele – HLA-DRB1\*03:01, -DRB1\*13:01 and/or -DRB1\*15:01 – and five patients were either heterozygous or homozygous for the HLA-B\*08:01 PSC risk allele (**Supplementary Table 1**).

In the antigen-experienced memory T-cell repertoire, a rearranged TCR $\beta$  nucleotide sequence (nucleotide clonotype) is generally unique to each T-cell clone [20, 21]. Thus, to investigate whether gut-infiltrating and liver-infiltrating T cells in human PSC-IBD are clonally related, we studied the overlap of nucleotide clonotypes in paired gut and liver samples from patients with PSC-IBD. An average clonotype overlap ranging from 9.1% (range: 6.4–13.5%) for the total TCR $\beta$  repertoire to 16.6% (range: 4.0–32.9%) for the nucleotide clonotypes present at frequencies greater than 0.1% was detected (**Fig. 1A**).

Naïve T cells can share the same TCR $\beta$  nucleotide sequence but have different TCR $\alpha$  sequences [20]. Therefore, in order to omit TCR $\beta$  nucleotide clonotypes that might originate from naïve T cells [22-25] and to focus on the more expanded clonotypes (here designated as ‘memory TCR $\beta$  repertoire’) we used a clonotype frequency of 0.01% as a threshold for further analysis.



Moreover, as data from circulating T cells might contaminate the TCR $\beta$  repertoires of gut- and liver-infiltrating T cells, any liver or gut nucleotide clonotype that was also detected in the matched blood sample at frequencies greater than 0.01% was excluded from the analysis. On average, an overlap of 9.7% (range: 4.7–19.9%) nucleotide clonotypes in the gut and liver memory TCR $\beta$  repertoire of each PSC-IBD patient was detected (**Fig. 1B**).

The overlapping nucleotide clonotypes of paired gut and liver memory TCR $\beta$  repertoires ('overlapping gut-liver memory clonotypes'), their encoded amino acid clonotypes, V and J genes and frequencies are given in **Supplementary Table 4**. A similar V and J gene usage of the overlapping gut-liver memory clonotypes and the gut and liver memory TCR $\beta$  repertoires was observed (**Supplementary Fig. 1–6** and **Supplementary Table 5–6**). An average of 16.0% (range: 8.7–32.6%) of liver memory T cells and 15.0% (range: 5.9–26.3%) of gut memory T cells carried overlapping gut-liver memory clonotypes (**Fig. 2**). Among the overlapping gut-liver memory clonotypes identified in this study, we detected amino acid clonotypes previously reported to recognize epitopes of polyomavirus BK (CASSLGGSNQPQHF), cytomegalovirus (CASSPSRNTEAFF, CASSPQRNTEAFF) and Epstein-Barr virus (CASSQSPGGTQYF, CSARDRVGNGYTF, CASSSLNTEAFF) [26-31]. Our data confirm the presence of T cells of common clonal origin in the liver and gut of patients with PSC-IBD.

*The overlap of TCR $\beta$  repertoires in PSC-IBD affected gut and liver tissue is significantly higher than in normal gut and liver tissue*

Next, we investigated the overlap of nucleotide clonotypes in paired normal gut and liver tissue sampled from 10 patients with colon cancer that developed colorectal metastasis in their liver (**Table 1** and **Supplementary Table 2**).

The average number of sequencing reads sampled from normal liver and gut samples were 58,159 reads (range: 2,452–158,151 reads) and 80,265 reads (range: 2,799–391,195), respectively (**Supplementary Table 7**). In comparison, the average number of sequencing reads sampled from PSC-IBD affected liver and gut samples were 810,609 reads (range: 171,374–1,957,860 reads) and 569,428 reads (range: 291,815–858,580 reads), respectively (**Supplementary Table 3**). Since overlap analyses are sensitive to differences in sample size, we normalized by downsampling the PSC-IBD affected tissue samples and normal tissue samples to the same number of reads [32]. Five colon cancer controls (CNTR-1, CNTR-2, CNTR-4, CNTR-7 and CNTR-8) were excluded from the overlap analyses due to the low read count of either their gut or liver sample. Consequently, we randomly picked 20,264 reads (*i.e.* the size of the smallest remaining sample) from the total repertoire of each PSC-IBD sample and remaining control sample. An average overlap of 3.6% (range: 1.6–4.6%) nucleotide clonotypes was detected in the gut and liver TCR $\beta$  repertoires of paired normal tissue, whereas an average clonotype overlap of 8.7% (range: 5.3–11.9%) was detected for paired PSC-IBD affected gut and liver tissue (**Fig. 3A**).

Differences in V and J gene usage were observed between normal and PSC-IBD affected gut and liver TCR $\beta$  repertoires (**Supplementary Fig. 7**).

Despite having the same number of reads in each patient sample, there were approximately ten times the total number of nucleotide clonotypes in samples from patients with PSC-IBD compared to samples from colon cancer controls (**Supplementary Table 8**). Therefore, we further picked the 100 most abundant nucleotide clonotypes from each sample and repeated the clonotype overlap measurements. An average clonotype overlap of 3.8% (range: 1.0–6.0%) was observed for paired normal gut and liver tissue (**Fig. 3B**). In comparison, an average clonotype overlap of 16.6% (range: 3.0–28.0%) was detected for paired gut and liver samples from patients with PSC-IBD. Additional overlap indices (*i.e.* Jaccard index and overlap coefficient) are given in **Supplementary Table 8** and **Supplementary Table 9**. The random downsampling procedure and overlap measurements were repeated five times, with similar degree of overlap observed each time (data not shown). Taken together, these findings suggest that there is a significantly higher sharing of TCR $\beta$  repertoires between paired PSC-IBD affected gut and liver tissue compared to paired normal gut and liver tissue.

#### *PSC-associated amino acid clonotypes are present in Norwegian PSC patients*

We next investigated whether we could detect the eight previously reported PSC-associated amino acid clonotypes [17]. The PSC-associated amino acid clonotypes were present in up to 50% of the PSC-IBD liver samples, in up to 40% of the PSC-IBD gut samples and in up to 90% of the PSC-IBD blood

samples (**Table 2**). The PSC-associated amino acid clonotypes that were detected in blood samples were found at lower frequencies compared to those encoded by identical nucleotide clonotypes in the matched gut or liver sample (**Supplementary Table 10**). We further detected two PSC-associated amino acid clonotypes (CASSDTSGGADTQYF, CASSPGQGEGYEYF) in 10% of the normal liver samples and one PSC-associated amino acid clonotype (CASSLGSGANVLTF) in 10% of the normal gut samples (**Table 2** and **Supplementary Table 10**). Two of the overlapping gut-liver memory clonotypes encoded previously reported PSC-associated amino acid clonotypes (CASSELAGGPETQYF, CASSPGQGEGYEYF) (**Supplementary Table 4**). Collectively, the previously identified PSC-associated amino acid clonotypes were also detected in the Norwegian PSC-IBD study population.

#### *Clonal distribution of liver-, gut- and blood-derived T cells*

The clonal distribution of T cells from patients with PSC-IBD was evaluated. The nucleotide clonotypes detected at a frequency of >0.01% constituted on average 63.6% and 60.2% of the total liver and gut TCR $\beta$  repertoires, respectively (**Fig. 4A**). The majority of nucleotide clonotypes in the PSC-IBD affected gut and liver samples had a frequency within a range of 0.001–0.01%, whereas an average of 86.4% of the nucleotide clonotypes detected in blood had a frequency of <0.001% (**Fig. 4B**). When assessing clonality, a measurement of T-cell diversity considering the variation of clonotype frequencies independent of sampling depth, similar scores for PSC-IBD affected liver (median 0.16, range 0.12–0.25), gut (median 0.15, range 0.08–0.35) and blood (median 0.20, range 0.03–0.46) TCR $\beta$  repertoires were

observed (**Fig. 4C**). Similar clonality scores were likewise observed for normal liver (median 0.12, range 0.08–0.38) and gut (median 0.10, range 0.06–0.20) TCR $\beta$  repertoires (**Supplementary Fig. 8**). These data suggest that there is a similar T-cell diversity in PSC-IBD affected tissues and in peripheral blood.

## DISCUSSION

The present study is the first to examine the T-cell repertoires of paired liver and gut samples using high-throughput sequencing of the TCR $\beta$  chain. We demonstrate for the first time the presence of human gut and liver memory T cells of common clonal origin. We observed a significantly higher sharing of TCR $\beta$  repertoires in paired PSC-IBD affected gut and liver samples than in paired tumor-adjacent normal gut and liver tissue, and further detected the previously reported PSC-associated amino acid clonotypes in the Norwegian population of PSC patients.

Our study provides the first human-based evidence that gut and liver memory T cells of common clonal origin are present in PSC patients. An average of 16% of liver memory T cells and 15% of gut memory T cells carried overlapping nucleotide clonotypes, suggesting that a high proportion of the memory T cell repertoire in gut and liver is able to recognize the same antigens or antigens that share sequence or structural similarities. Our data are compatible with the hypothesis that an enterohepatic migration of T cells contributes to the pathogenesis of PSC, as previously suggested based on the aberrant expression of gut-specific molecules, the endothelial adhesion molecule MAdCAM-1 and chemokine CCL25, and the presence of  $\alpha 4\beta 7^+ \text{CCR9}^+$  effector memory T cells in the inflamed livers of PSC patients [7-9]. The shared gut and liver TCR $\beta$  repertoires might also be the result of shared naïve T-cell clones and antigens occurring at both sites, which in the presence of shared HLA haplotypes led to activation and expansion of the same naïve T-cell clones in the gut and the liver. Nevertheless, the significantly higher sharing of TCR $\beta$  repertoires between paired PSC-IBD

affected tissues than between paired tumor-adjacent normal gut and liver tissue suggests that our findings are related to PSC-IBD pathogenesis.

Previously, we had identified eight amino acid clonotypes that were present in 30-40% of UK PSC livers (n=20) and absent from liver tissue sampled from patients with primary biliary cirrhosis (n=10) and alcoholic liver disease (n=10) [17]. In the current study, we detected these PSC-associated amino acid clonotypes in up to 50% of the liver samples and up to 40% of the gut samples from Norwegian patients with PSC-IBD.

There are several constraints to be acknowledged in the present study. The availability of only fresh-frozen or FFPE gut and liver samples from patients with PSC-IBD and from colon cancer controls, respectively, did not allow us to sort the T cells prior to sequencing. The use of sorted T cell subsets might reveal more detailed information on the clonotype overlap between the two compartments, however this would require the isolation of cells at the time of each sample collection. We further acknowledge that differences in TCR $\beta$  repertoire overlap might exist across populations from different geographical origins, as the normal tissue samples and the PSC-IBD affected tissue were sampled in the UK and in Norway, respectively. Moreover, gDNA extracted from FFPE tissue is likely fragmented and this might have affected the number of sequencing reads retrieved from the colon cancer control samples, however fragmentation is unbiased and thus will not specifically affect particular nucleotide clonotypes. Finally, we acknowledge that the PSC-IBD affected liver samples and colonic biopsies used in our study represent only a small proportion of the diseased microenvironment. This, in addition to the

patchy nature of PSC disease, makes the task of identifying overlapping clonotypes in PSC-IBD challenging.

The colonic biopsies and explanted livers were sampled from the ten patients with PSC-IBD over a time period of more than 7 years. Utilizing this rare collection of matched PSC-IBD samples and the highly sensitive high-throughput sequencing approach, human gut- and liver-infiltrating T cells of common clonal origin could be identified for the first time. A similar study has not been previously feasible due to the constraints of older technologies and the limited access to matched material from PSC patients. We are unable to confirm whether the T cells were initially activated in the gut, liver or elsewhere. Sampling each tissue at multiple time points (ideally tracking clonotypes before the onset of disease) would have been required. This is, however, ethically inappropriate and thus impossible.

In conclusion, we have detected the presence of previously reported PSC-associated amino acid clonotypes in a Norwegian PSC-IBD study population, and provide the first evidence that gut and liver memory T cells of common clonal origin are present in patients with PSC-IBD. Our data suggest this is related to PSC-IBD pathogenesis, supporting the hypothesis that memory T cells with shared antigenic drivers are present in the gut and liver of PSC patients. This mechanism is likely to be biologically important and represents a potential therapeutic target for patients with concurrent PSC and IBD.



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Author names in bold designate shared co-first authorship.

**Table 1. Characteristics of the study populations.**

Plus or minus values are mean  $\pm$  standard deviation.

<sup>a</sup> Mean INR values from 6 of 10 colon cancer patients at time of colectomy and liver resection

<sup>b</sup> Mean ALT values from 8 and 9 of 10 colon cancer patients at time of colectomy and liver resection, respectively

<sup>c</sup> Mean platelets values from 7 and 9 of 10 colon cancer patients at time of colectomy and liver resection, respectively

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; IBD, inflammatory bowel disease; INR, international normalized ratio; PSC, primary sclerosing cholangitis

Characteristics	PSC-IBD (n=10)		Colon cancer control (n=10)	
	at colonoscopy	at liver transplantation	at colectomy	at liver resection
Age, years	44 $\pm$ 11	47 $\pm$ 10	65 $\pm$ 10	65 $\pm$ 9
Male sex, %	60	60	40	40
Bilirubin, umol/L	25 $\pm$ 15	16 $\pm$ 10	11 $\pm$ 6	13 $\pm$ 10
INR <sup>a</sup>	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2
ALT, U/L <sup>b</sup>	190 $\pm$ 156	86 $\pm$ 79	70 $\pm$ 87	131 $\pm$ 84
ALP, U/L	318 $\pm$ 259	231 $\pm$ 126	181 $\pm$ 105	96 $\pm$ 41
Albumin, g/L	39 $\pm$ 6	40 $\pm$ 6	35 $\pm$ 10	30 $\pm$ 9
Platelets, 10 <sup>9</sup> /L <sup>c</sup>	298 $\pm$ 101	293 $\pm$ 125	298 $\pm$ 104	215 $\pm$ 65

**Table 2. Primary sclerosing cholangitis (PSC) associated amino acid clonotypes.** The table shows the number of samples in which each of the previously reported PSC-associated amino acid clonotypes [17] was observed in Norwegian patients with concurrent PSC and inflammatory bowel disease (IBD) and in tumor-adjacent normal tissue of colon cancer patients from Birmingham, UK.

PSC-associated amino acid clonotypes	Number of Norwegian PSC-IBD			Number of control	
	liver samples, n=10	gut samples, n=10	blood samples, n=10	liver samples, n=10	gut samples, n=10
CASSDTSGGADTQYF	1	0	2	1	0
CASSELAGGPETQYF	1	4	3	0	0
CASSEYSNQPQHF	1	1	3	0	0
CASSFTGTDTQYF	0	1	3	0	0
CASSGTSGGADTQYF	3	1	2	0	0
CASSLGSGANVLTF	5	2	9	0	1
CASSPGQGEGYEYF	2	1	3	1	0
CASSPPSYEQYF	1	2	8	0	0



## Figure Legends

**Fig. 1. Clonotype overlap in paired gut and liver samples from patients with primary sclerosing cholangitis-inflammatory bowel disease.** For each patient, a T-cell receptor beta repertoire overlap was calculated based on the number of nucleotide clonotypes found in both gut and liver. (A) Data show the overlap of gut and liver clonotypes according to their frequencies. (B) Data show each patient's overlap of gut and liver nucleotide clonotypes found at a frequency of  $>0.01\%$ . Excluded from the analysis was any gut or liver nucleotide clonotype that was also detected in the matched blood sample at a frequency of  $>0.01\%$ .

**Fig. 2. Percentage of gut and liver memory T cells carrying overlapping gut-liver memory clonotypes in primary sclerosing cholangitis.** Data show the proportion of each patient's gut and liver memory T cells carrying clonotypes that are overlapping between gut and liver memory T-cell receptor beta ( $\text{TCR}\beta$ ) repertoires ('overlapping gut-liver memory clonotypes') calculated as  $(\text{number of sequencing reads for overlapping gut-liver memory clonotypes}) / (\text{total number of sequencing reads for memory } \text{TCR}\beta \text{ repertoire}) \times 100$ . 'Memory  $\text{TCR}\beta$  repertoire' denotes here the clonotypes found at a frequency of  $>0.01\%$ , and which were not detected in the matched blood sample at a frequency of  $>0.01\%$ .

**Fig. 3. Clonotype overlap in normal and primary sclerosing cholangitis-inflammatory bowel disease (PSC-IBD) affected paired gut and liver tissue.** For each colon cancer control ( $n=5$ ) and PSC-IBD patient ( $n=10$ ), a T-cell receptor beta repertoire overlap was calculated based on the number of

nucleotide clonotypes found in both gut and liver sample. (A) Data show the overlap of gut and liver clonotypes in controls and PSC-IBD patients. Each sample contained 20,264 reads. (B) Data show the overlap of gut and liver clonotypes amongst the 100 most abundant nucleotide clonotypes in samples with 20,264 reads from controls and PSC-IBD patients. (\*p=0.0117, \*\*\*p=0.0007).

**Fig. 4. Clonal distribution of liver-, gut- and blood-derived T cells.** (A) Cumulative percentage distributions of nucleotide clonotypes at various frequencies in liver, gut and blood T-cell receptor beta (TCR $\beta$ ) repertoires of primary sclerosing cholangis-inflammatory bowel disease (PSC-IBD) patients. (B) Frequency distributions of the liver-, gut- and blood-derived nucleotide clonotypes of PSC-IBD patients. (C) Data show the clonality of liver, gut and blood TCR $\beta$  repertoires of PSC-IBD patients. Data points represent the clonality score of each patient's sample, and the horizontal lines represent the median of each group. Liver versus gut p=0.353; liver versus blood p=0.739; gut versus blood p=0.684.

Figure 1

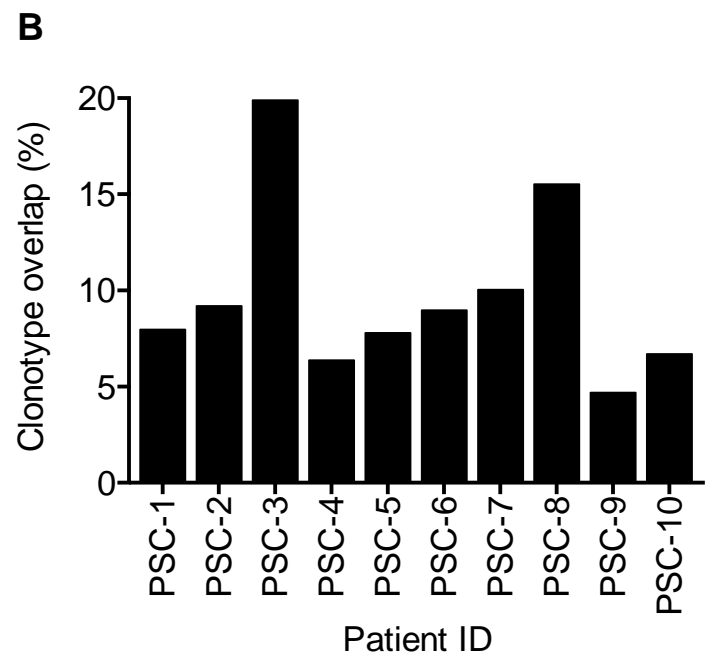
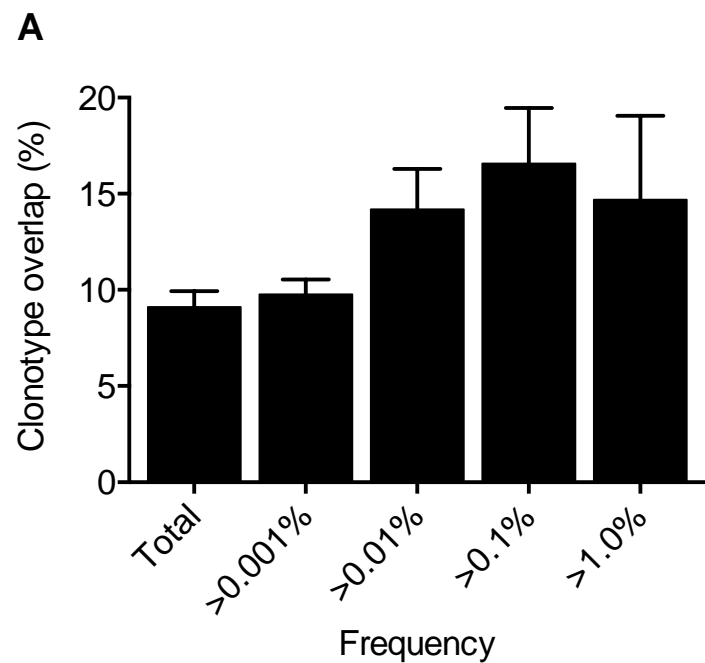


Figure 2

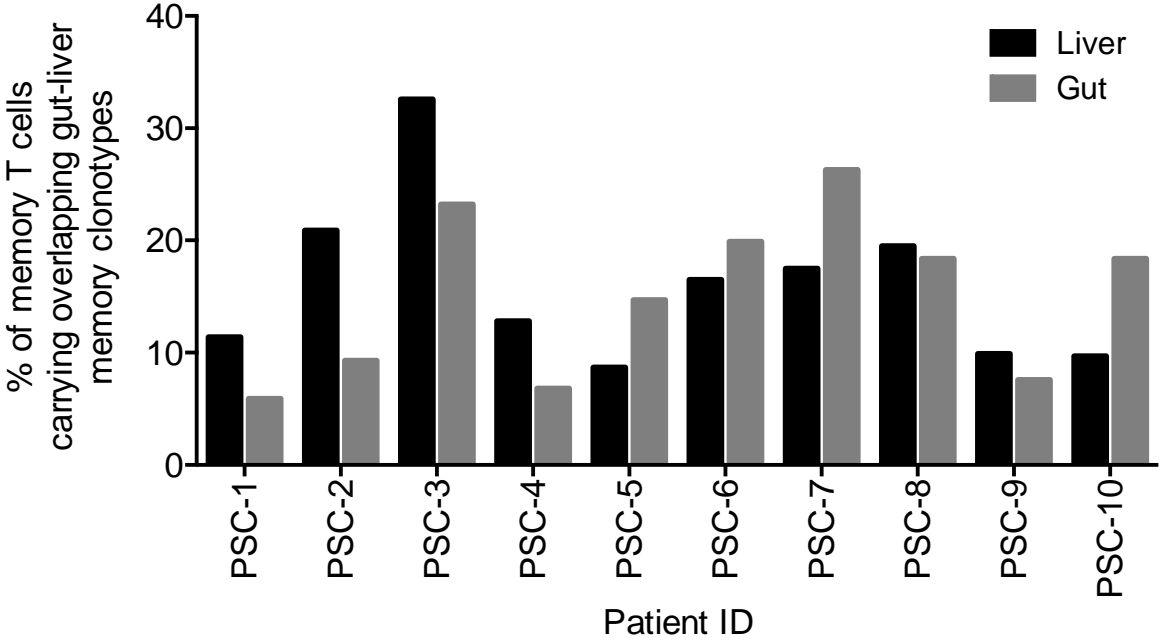
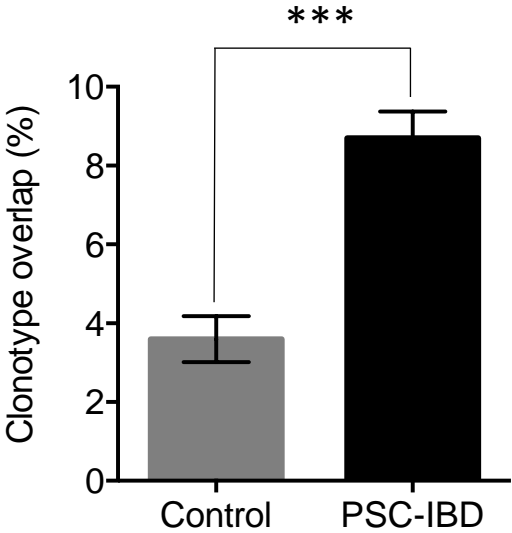


Figure 3

**A**



**B**

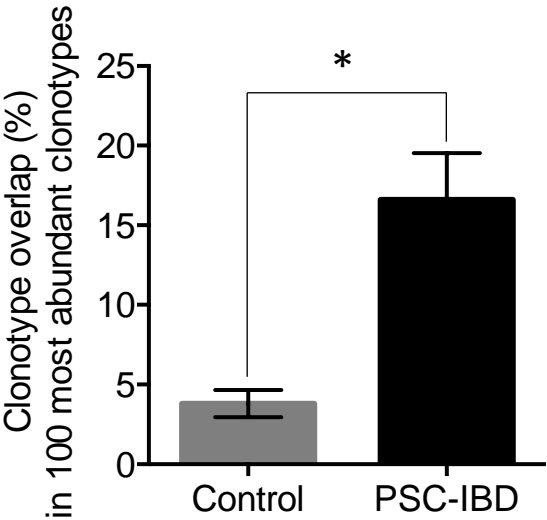
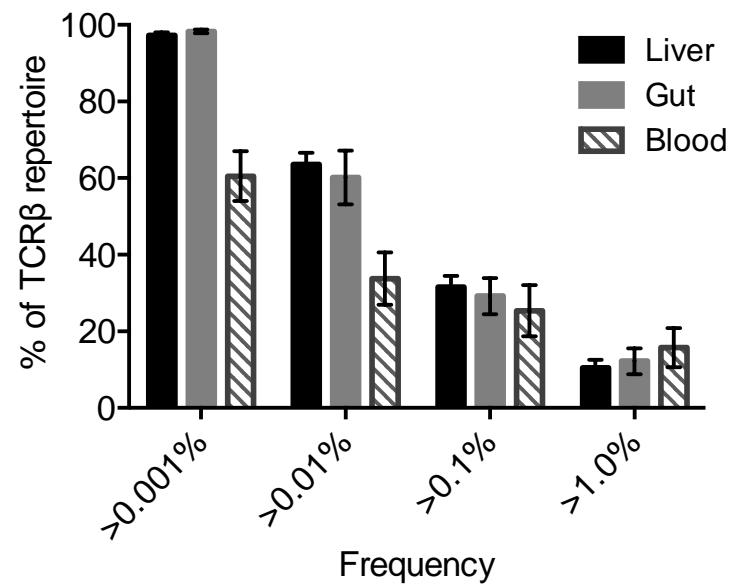
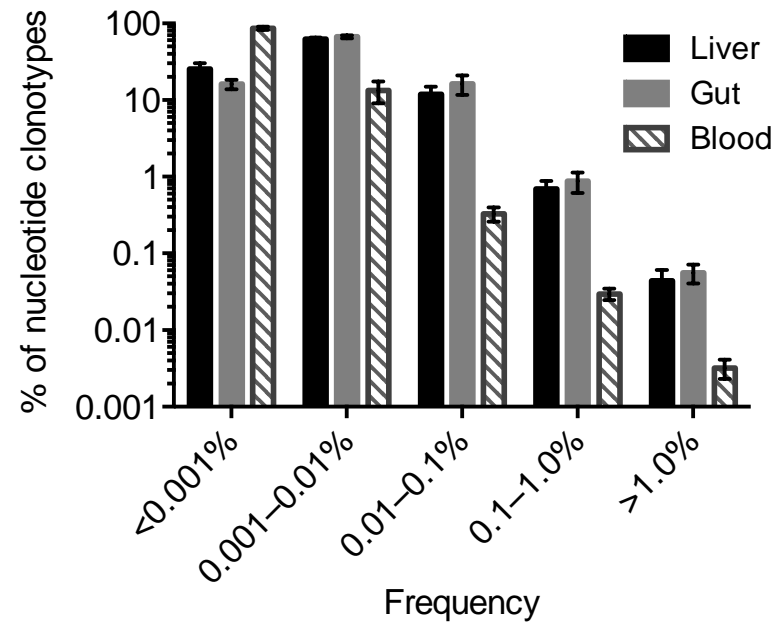


Figure 4

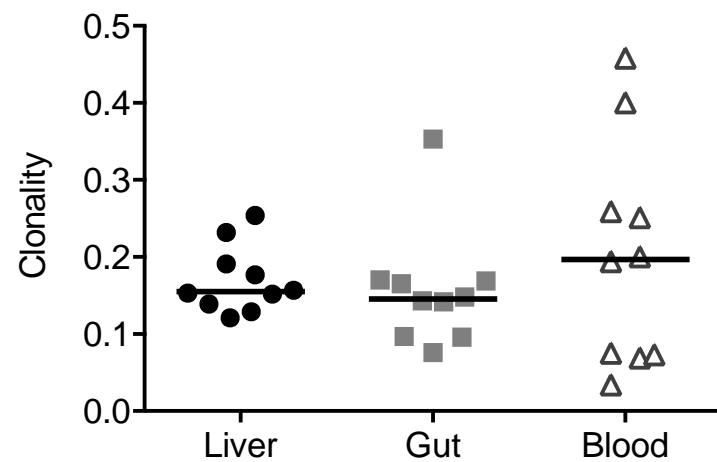
**A**

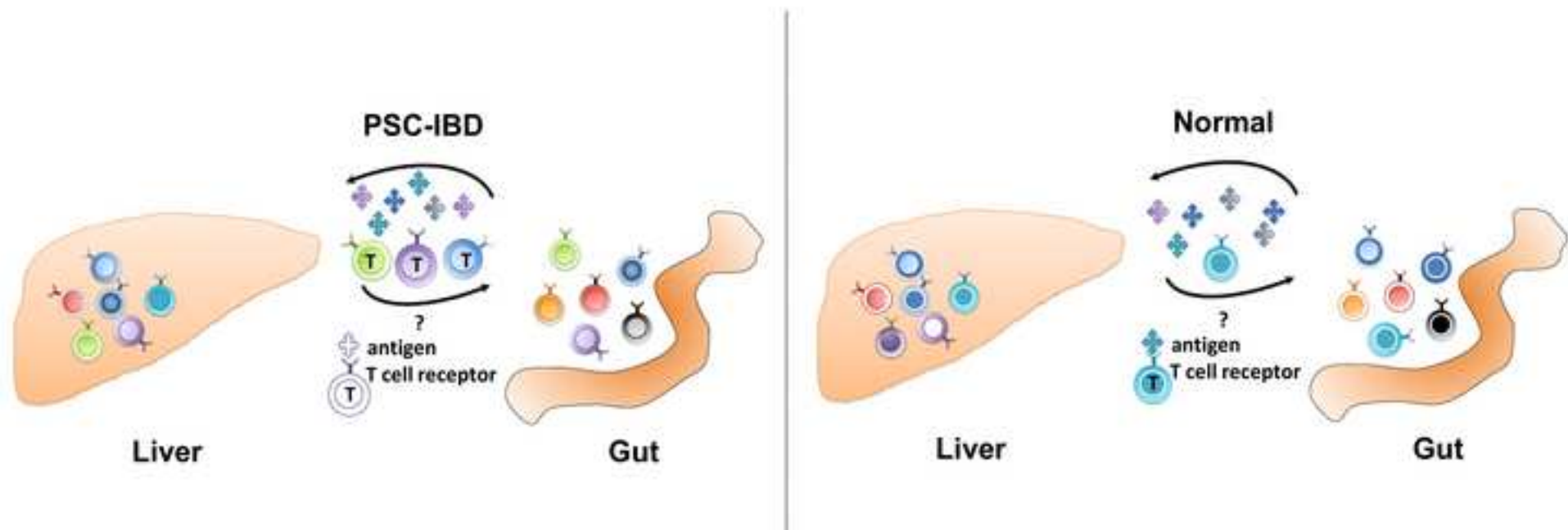


**B**



**C**





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